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Original Article

Studies on the extraction and purification of phytic acid from rice bran

Cristiane Canan [a], Felipe Tsuruta Lisboa Cruz [a], Fernanda Delaroza [a], Rubia Casagrande [b], Cleonice Pereira Mendes Sarmento [c], Massami Shimokomaki [a], Elza Iouko Ida [a,*]

[a] Londrina State University, Department of Food Science and Technology, Londrina, Paraná, Brazil
[b] Londrina State University, Department of Pharmaceutical Sciences, Londrina, Paraná, Brazil
[c] Federal Technological University of Paraná, Campus Medianeira, Department of Food Technology, Medianeira, Paraná, Brazil

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ABSTRACT

The objective of this work was to develop rice bran IRGA 417 phytic acid (PA) extraction and purification techniques. For the extraction of PA, a complete 2^4 factorial design with triplicates at the central point was used, and the effects of concentration of rice bran and HCl, time and temperature were investigated. During purification, different pH values were tested with addition of 1.5 M Na_2CO_3 or 4.0 M NaOH. The results obtained by the statistical analysis of the factorial design showed that temperature, time and HCl concentration influenced the PA extraction technique significantly ($p \leq 0.05$), whereas the concentration of rice bran had no influence. The content of PA was evaluated in all the stages of purification and it was possible to establish an improved methodology of extraction and purification with high purity and yields.

1. Introduction

Phytic acid (PA) or myo-inositol hexaphosphate is a strong chelator of multivalent metal ions, especially iron, zinc and calcium (Hurrel, 2004). For many years, PA was considered an anti-nutritional compound because it reduces the bioavailability of several minerals important for human nutrition (Bohn et al., 2007; Li et al., 2008; Schelemmer et al., 2009). However, since the 1990s, PA has been scientifically emphasized for its beneficial effects on human health, particularly in the prevention of diabetes (Lee et al., 2006), renal calculi (Saw et al., 2007), Parkinson's disease (Xu et al., 2008) and cancer (Vucenik and Shamsuddin, 2006). The application of 0.50% PA acid rice bran added to the drinking water after tumor induction, reduced the risk of colon cancer in rats (Norazalina et al., 2009). Its antioxidant effect, described by several researchers (Lee and Hendricks, 1995; Soares et al., 2004; Stodolak et al., 2007; Harbach et al., 2007), is due to its ability to inhibit the formation of hydroxyl radicals ($^{\bullet}HO$) and to form chelates with Fe^{2+} ions, causing them to become catalytically inactive (Graf and Eaton, 1990).

PA also significantly inhibits the development of warmed over flavor (WOF) in beef (Lee et al., 1998) and chicken meat (Shimokomaki et al., 1999). It prevents lipid oxidation in a synergic manner, as measured in chicken meat, while the birds received vitamin E in their diets (Soares et al., 2004). Recently, our laboratory demonstrated that PA prevented rancidity in meat without endangering health when administered in a pig's diet (Harbach et al., 2007). In addition, the antioxidant property of PA obtained from corn germ was also assessed, and its potential antioxidant role was confirmed through deoxyribose and bathophenanthroline tests (Filgueiras et al., 2009).

PA is distributed in the different rice components, primarily concentrated in the germ with 7.6 g 100 g^{-1} and endosperm with 1.2 g 100 g^{-1} (O'Dell et al., 1972). Rice bran, consisting of pericarp, aleurone and germ, has a high concentration of PA ranging from 5.94 to 6.09 g 100 g^{-1} (Kasim and Edwards, 1998). However, the PA content ranges depending on the local rice cultivation conditions (Liu et al., 2005). Rice bran is considered a rice industry by-product and corresponds to 10 g 100 g^{-1} of integral rice grain. It is used for oil extraction, feed production and also as an ingredient in the formulation of food products because of its protein, lipid, mineral and antioxidant contents (Parrado et al., 2006).

Rice bran is a by-product of rice industry and produced on a large scale, with high levels of PA that presents antioxidant capacity and potential beneficial effects on health. Therefore, this work aimed to describe an analytical technique for the efficient extraction and purification of PA using rice bran as a model system.

* Corresponding author. Fax: +55 43 3371 4080.
E-mail address: elida@uel.br (E.I. Ida).

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2. Materials and methods

2.1. Materials and reagents

IRGA 417 cultivar raw rice bran was kindly donated by the Rio Grande Rice Institute (IRGA), Cachoeirinha/Rio Grande do Sul, Brazil, harvest 2007/2008. The amount donated was 10 kg and kept under refrigeration throughout the experiment. All experiments were done with single lot. Dodecasodium phytate from rice (P0109/Sigma, St. Louis, USA) was used as a standard and all chemicals used were of analytical grade.

2.2. Phytic acid determination

PA was extracted with 0.8 M HCl at a concentration of 0.1 g mL^{-1} and quantified according to the procedure described by Latta and Eskin (1980) based on the reaction between ferric ion and sulfosalicylic acid with modification of the resin to Dowex-AGX-4, as proposed by Ellis and Morris (1986). The result was expressed as g of PA in 100 g of sample or 100 mL of extract.

2.3. Experimental design for extraction of phytic acid from rice bran

To investigate the technique of PA extraction, the following independent variables were selected: X_1 (rice bran concentration, g mL^{-1}), X_2 (hydrochloric acid concentration, M), X_3 (temperature, °C) and X_4 (time, h), with three levels of variation (−1, 0 and +1). Table 1 shows the matrix of the experimental design (2^4) with the levels of variation and the dependent variable or Y (experimental response) and the $\hat{Y}$ (estimated response), expressed as g of PA extracted in 100 g of rice bran, on a dry basis. The assays were carried out at random. The software STATISTICA 7.0 was used for regression analysis and to create a surface response model. The model is expressed as Eq. (1):

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 + e \quad (1)$$

where Y = response, x_1, x_2, x_3 and x_4 = coded variables, β = estimated coefficients in the response surface model, e = residual (experimental error).

After examining the response surface was conduct in triplicate assays under the optimum conditions to experimental to validation of the proposed model.

2.4. Development of the phytic acid purification technique

An analytical procedure for the purification of PA (Fig. 1) was developed after establishing the conditions according to experimental design (Section 2.3) of extraction for 1 h at 25 °C (shaker model MA 830/A, Marconi, São Paulo, Brazil) with 1.0 N HCl and a concentration of rice bran of 0.1 g mL^{-1}. The pH of the extract containing PA was adjusted to 4.5 with a solution of 4 M NaOH, the rice protein's isoelectric point (Gupta et al., 2008), and the extract was centrifuged at 2000 × g for 10 min (model BR4i, Jouan, Saint Herblain, France). A supernatant (S_1) and pellet (P_1) were obtained.

2.4.1. Effect of pH on phytic acid precipitation

In S_1, the best pH conditions for precipitating and reducing the losses of PA were found with a solution of 1.5 M Na_2CO_3 or 4 M NaOH and varying the pH value from 7.0 to 9.0 in increments of 0.5. After adjusting the pH of the respective supernatant S_1, the solution was store for an additional 12 h. The formed pellets were recovered by centrifugation, as discussed above, and the supernatants were discarded. Ten pellets (P_2) were obtained: 5 from the precipitation with 1.5 M Na_2CO_3 and 5 from the precipitation with 4 M NaOH. The P_2 pellets containing PA were resuspended with 1 M HCl, 10 mL of formaldehyde and 0.5 g of diatomaceous earth to denature the proteins and remove other contaminants. The suspensions were shaken for 2 h at ambient temperature in a hood, kept at rest for an additional 12 h and filtered through qualitative filter paper Whatman # 3. In the filtered solution (S_3) the best pH condition for PA precipitation was found with a 1.5 M Na_2CO_3 solution and varying the pH value from 7.0 to 9.0 in increments of 0.5. After adjusting the pH of S_3, the pellet formed (P_4) was recovered through qualitative filter paper Whatman # 3, and the filtered solution was discarded. All of the recovered PA precipitates were dried for 24 h in an oven at 60 °C and the content and yield of the purified PA were determined.

Table 1
Planning assay 2^4 matrix with independent variables, experimental responses (Y) and estimated responses ($\hat{Y}$) of phytic acid (PA) extracted from rice bran.

Assays	Coded (real)				g of extracted PA 100 g^{-1} of rice bran	
	Rice bran (g mL^{-1})	HCl (M)	Temperature (°C)	Time (h)	Y[a]	$\hat{Y}$
	x_1 (X_1)	x_2 (X_2)	x_3 (X_3)	x_4 (X_4)		
1	−1 (0.05)	−1 (0.2)	−1 (25)	−1 (1)	4.96 ± 0.12	4.92
2	1 (0.10)	−1 (0.2)	−1 (25)	−1 (1)	4.96 ± 0.21	5.00
3	−1 (0.05)	1 (1.0)	−1 (25)	−1 (1)	7.08 ± 0.25	7.01
4	1 (0.10)	1 (1.0)	−1 (25)	−1 (1)	5.88 ± 0.09	5.96
5	−1 (0.05)	−1 (0.2)	1 (75)	−1 (1)	3.53 ± 0.89	3.51
6	1 (0.10)	−1 (0.2)	1 (75)	−1 (1)	4.69 ± 0.37	4.72
7	−1 (0.05)	1 (1.0)	1 (75)	−1 (1)	4.34 ± 0.19	4.48
8	1 (0.10)	1 (1.0)	1 (75)	−1 (1)	4.69 ± 0.12	4.55
9	−1 (0.05)	−1 (0.2)	−1 (25)	1 (3)	4.89 ± 0.25	4.94
10	1 (0.10)	−1 (0.2)	−1 (25)	1 (3)	4.51 ± 0.07	4.46
11	−1 (0.05)	1 (1.0)	−1 (25)	1 (3)	6.96 ± 0.05	7.02
12	1 (0.10)	1 (1.0)	−1 (25)	1 (3)	5.47 ± 0.12	5.41
13	−1 (0.05)	−1 (0.2)	1 (75)	1 (3)	3.35 ± 0.22	3.37
14	1 (0.10)	−1 (0.2)	1 (75)	1 (3)	4.04 ± 0.62	4.01
15	−1 (0.05)	1 (1.0)	1 (75)	1 (3)	4.47 ± 0.23	4.33
16	1 (0.10)	1 (1.0)	1 (75)	1 (3)	3.70 ± 0.22	3.83
17	0 (0.075)	0 (0.6)	0 (50)	0 (2)	5.05 ± 0.15	4.97
18	0 (0.075)	0 (0.6)	0 (50)	0 (2)	4.67 ± 0.76	4.97
19	0 (0.075)	0 (0.6)	0 (50)	0 (2)	5.19 ± 0.35	4.97

[a] Mean ± standard deviation (n = 3).

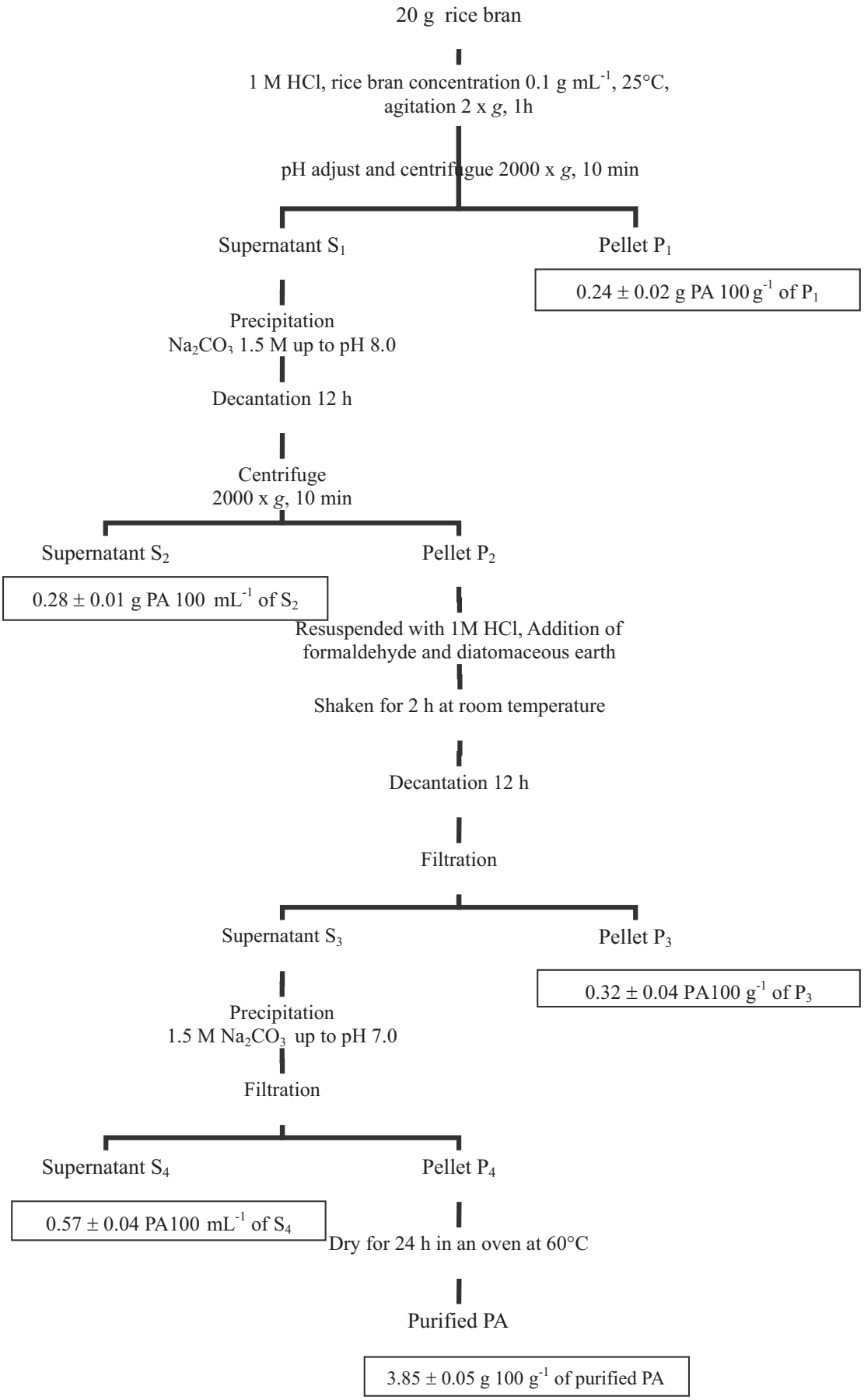

Fig. 1. Fluxogram for extraction and purification of rice bran phytic acid (PA).

2.5. Purified phytate yield

The content of PA was determined according to Section 2.2. The yield was expressed as a percentage and calculated as the ratio of the initial rice bran PA content (PA_i) to the obtained PA content (PA_o), multiplied by 100.

2.6. Process evaluation of phytic acid purification

2.6.1. Content of organic, inorganic and total phosphorus

The extraction and digestion of organic phosphorus, P, from the PA standard and the rice bran were performed according to the method described by Thompson and Erdman (1982) and colorimetrically determined according to Chen et al. (1956). The inorganic P was analyzed from the supernatant obtained after PA extraction and quantified in the same manner as the total P: by optical emission spectrometry with an inductively coupled plasma source (ICP-OES) (model Optima 3000 DV, Perkin Elmer), according to Sousa et al. (2005).

2.6.2. Soluble protein, total nitrogen and total protein contents

The quantification of the soluble protein in the PA standard and the purified PA was conducted according to Lowry et al. (1951). The quantification of the total nitrogen and the total protein (factor 6.25) was performed according to the Association of Official Analytical Chemists (AOAC, 2005).

2.6.3. Mineral contents

The quantification of Cu, Zn, Ni, Co, Mn, Fe, Ca and Mg in the standard and the purified PA was carried out in the ICP-OES, (model Optima 3000 DV, Perkin Elmer, Norwalk, CT, USA) according to Sousa et al. (2005). The content of Na and K was determined with a flame photometer (model B462, Micronal, São Paulo, Brazil), according to Dean (1960).

2.6.4. Solubility

The purified rice bran PA solubility was investigated at pH 4.0, 6.0 and 8.0 in disodium phosphate and a citric acid buffer. For each pH value, purified PA in a saturated acid solution was prepared and shaken for 12 h, followed by filtration through filter paper Whatman # 3 according to Casagrande et al. (2007). PA was quantified in the filtrates according method of Section 2.2.

2.7. Statistical analysis

The statistical analysis (ANOVA) was carried out using the program STATISTICA 7.0 (Statsoft Inc. Corporate Tulsa, OK, EUA) (Statsoft, 2004) followed by Tukey test. The results were expressed as the mean ± standard deviation (SDM) and considered significantly different at $p \leq 0.05$.

3. Results and discussion

3.1. Phytic acid extraction technique

Tables 1–3 present the effects of the variables and the response estimated by the model ($\hat{Y}$) for the IRGA 417 rice bran PA extraction. According to the regression coefficients (Table 2), the variables X_2 (concentration of HCl), X_3 (temperature) and X_4 (time) had a significant effect on the response (Y). These results were similar to those for soya PA extraction reported by Han (1988) and for extruded cotton and rice bran by Fuh and Chiang (2001). According to Kolchev (1978) the important variables for the production of rice bran phytin were a solid/liquid ratio of 1:5, a concentration of 0.1 M HCl and an extraction time of 2 h. There are few descriptions in the literature of the extraction of PA from grains for the purpose of purification, although many studies have been performed on extracting and quantifying the amount of PA.

For PA extraction in legumes and cereals, either water or an acid solvent is routinely used (Kolchev, 1978). Hydrochloric acid is most often employed in concentrations ranging from 0.01 to 2.4 M (Makower, 1970; Han, 1988; Park et al., 2006). Other acids have also been used in different concentrations such as phosphoric, acetic, sulfuric (Han, 1988), perchloric (Makower, 1970) and trichloroacetic acids (Uppström and Svensson, 1980).

According to Han (1988), there is no clear relationship between strong and weak acids in the extraction of PA in soy and cottonseed samples. For the extraction of PA from different raw materials, temperature is also considered an important factor. Various temperatures have been used: 20 °C (Han, 1988; Fuh and Chiang, 2001), 8 °C (Bohn et al., 2007) and 25–50 °C (Han, 1988). In this investigation, the variable X_1 (rice bran concentration) and the interactions X_2X_4 and X_3X_4 showed no significant effects. Depending on the sample, the concentration of rice bran using the solid–liquid ratio (w/v) was also considered one of the factors. The following ratios have been used in the literature: 1:3 (Park et al., 2006), 1:10 (Bos et al., 1991), 1:15 (Makower, 1970), 1:20 (Latta and Eskin, 1980) and 1:25 (Uppström and Svensson, 1980).

The lowest level of PA was extracted under the following conditions: 0.2 M HCl ($x_2 = -1$), temperature of 75 °C ($x_3 = +1$) and extraction time of 3 h ($x_4 = +1$). The assays reproduced the proposed model as well as confirmed by the low relative standard error (0.48) for Tests 17, 18 and 19 (Table 1).

Taking into consideration the fact that the variance analysis and regression were significant (Tables 2 and 3), three mathematical models were developed using predictive variables coded for the significant responses X_2, X_3 and X_4 The model (Eq. (2)) that best represented the process of PA extraction contained only the significant variables, such as X_2 (concentration of HCl), X_3 (temperature) and the interaction between X_2 and X_3. The

Table 2
Regression coefficients for response Y = g of extracted phytic acid 100 g^{-1} of sample.

Variation source	Regression coefficients	Standard error	t (45)	p-value
Mean	4.9739	0.1182	42.0734	0.0000*
(X_1) Rice bran concentration (L)	−0.1026	0.1024	−2.0034	0.0512
(X_1) Rice bran concentration (Q)	−0.1288	0.2577	−0.9996	0.3229
(X_2) HCl concentration (L)	0.4786	0.1024	9.3493	0.0000*
(X_3) Temperature (L)	−0.7441	0.1024	−14.5365	0.0000*
(X_4) Time (L)	−0.1724	0.1024	−3.3686	0.0016*
(X_1) × (X_2)	−0.2847	0.1024	−5.5612	0.0000*
(X_1) × (X_3)	0.2798	0.1024	5.4656	0.0000*
(X_1) × (X_4)	−0.1408	0.1024	−2.7514	0.0085*
(X_2) × (X_3)	−0.2804	0.1024	−5.4777	0.0000*
(X_2) × (X_4)	−0.0030	0.1024	−0.0582	0.9538*
(X_3) × (X_4)	−0.0409	0.1024	−0.7983	0.4289*

Table 3
Linear ANOVA model for predicting the extraction of rice bran phytic acid.

Source of variation	SQ[a]	GL[b]	QM[c]	$F_{calculated}$
Regression	52.0867	10	5.2087	42.3298
Residuals	5.6603	46	0.1231	
Total	57.7470	56		

% explained variation (R^2) = 90.20 ($F_{0.05,\ 10.46}$ = 2.05).
[a] Sum of squares.
[b] Degrees of freedom.
[c] Mean squares.

nonsignificant parameters were incorporated into the residuals to calculate the analysis of variance (ANOVA) (Table 3).

$$Y = 4.97 + 0.48x_2 - 0.74x_3 - 0.28x_2x_3 \quad (2)$$

From the results, it was possible to construct the surface response (Fig. 2), and the variables X_2 (concentration of HCl) and X_3 (temperature) were individually evaluated. As it can be observed, there was an optimal region for maximum extraction of rice bran PA in the region of 0.72–1.8 M HCl (X_2) and for a temperature (X_3) of around 25 °C.

Before validating the expected response of the predictive model, the extraction times (X_4) of PA of 30 min and 45 min were tested in triplicate and compared with the extraction time of 1 h. We observed that the extraction time of 1 h differed significantly ($p \leq 0.05$) from the time of 30 min and 45 min. The content of extracted PA in 100 g of rice bran on a dry basis for these three extraction times was 5.88 ± 0.09, 4.55 ± 0.17 and 2.86 ± 0.14 g, respectively. Thus, it was determined that the maximum extraction of PA occurred when the minimum time was 1 h ($X_4 = -1$), a concentration of 1 M HCl ($x_2 = +1$) was used, the concentration of rice bran was maintained of at 0.1 g mL^{-1} ($x_1 = -1$) and the temperature was 25 °C ($x_3 = -1$). After confirming these conditions as optimum for the extraction of rice bran PA, an assay was performed in triplicate. The response (Y) was 6.48 g of extracted PA in 100 g of rice bran on a dry basis. Replacing X_2, X_3 and the X_2X_3 interaction in Eq. (2), we obtained the expected response of 6.70 g of extracted PA in 100 g of rice bran on a dry basis. The predicted relative error of the model ($\hat{Y}$) in relation to the PA content was 3.28%. These results confirm the validity of the predictive model and show that the data were properly adjusted to the experimental data.

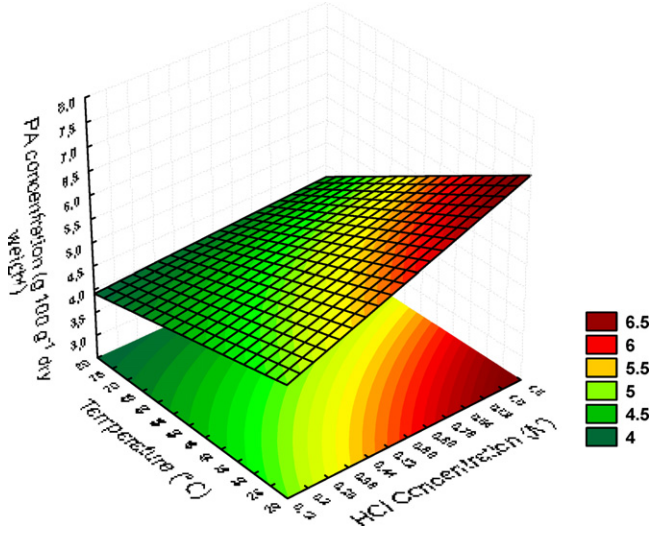

Fig. 2. Surface response model for extraction of rice bran phytic acid as a function of X_2 (HCl concentration) and X_3 (temperature).

Table 4
Phytic acid contents of the supernatant S_2 with adjustments of pH with 1.5 M Na_2CO_3 or 4 M NaOH.

Supernatant S_2 pH	Phytic acid content (g.100 mL^{-1})	
	1.5 M Na_2CO_3 Solution	4 M NaOH Solution
7.0	$0.79^{aB} \pm 0.14$	$1.41^{aA} \pm 0.21$
7.5	$0.44^{bB} \pm 0.08$	$0.78^{bA} \pm 0.04$
8.0	$0.28^{bcB} \pm 0.01$	$0.60^{bA} \pm 0.01$
8.5	$0.17^{cB} \pm 0.02$	$0.24^{cA} \pm 0.01$
9.0	$0.15^{cA} \pm 0.01$	$0.12^{cA} \pm 0.02$

Mean ± SD followed by different lowercase letters in the same column differ by Tukey test ($p \leq 0.05$). Mean ± standard deviation followed by capital letters in the same row differ by Tukey test ($p \leq 0.05$) ($n = 3$ replicates).

3.2. Analytical procedures for phytic acid purification

After validation of the predictive extraction method model, every step of the purifying analytical technique was verified, including the conditions in which they provided the maximum purity grade and the minimum loss (Fig. 1). The effect of pH on precipitation was investigated, and the PA content was determined in the supernatant S_2 (Table 4) after adjusting the pH with solutions of 1.5 M Na_2CO_3 or 4 M NaOH. The minimum loss occurred when the pH of supernatant S_2 was adjusted with 1.5 M Na_2CO_3 from 8.0 to 9.0 or with 4.0 M NaOH from 8.5 to 9.0. The PA content did not differ among groups ($p > 0.05$) when 1.5 M Na_2CO_3 or 4 M NaOH was used to adjust the pH value to 9.0. At this stage of the purification process, the lowest pH adjustment was chosen, pH 8.0 with 1.5 M Na_2CO_3, which did not change ($p > 0.05$) with a pH adjustment to 8.5. In his work on phytin preparation from rice bran and other wastes from food industries, Kolchev (1978) employed $NaHCO_3$ to adjust the pH of the acid extract to 7.0 and 8.0. Inorganic phosphorus (Na_2HPO_4) were discarded through precipitation because were formed the insoluble $Ca(HCO_3)_2$ and $Mg(HCO_3)_2$.

In the step for precipitating PA in supernatant S_3, only 1.5 M Na_2CO_3 was used, as described in the previous step for adjusting the pH from 7.0 to 9.0 at 0.5 pH value intervals. After filtering with filter paper Whatman # 3 and drying the pellet in an oven at 60 °C, a white powdery PA was obtained. The yield of purified PA (Table 5) at different pH values did not differ among groups ($p > 0.05$). The addition of 1.5 M Na_2CO_3 decreased the purity grade because larger volumes of 1.5 M Na_2CO_3 were used for the precipitation. The maximum degree of purity was obtained when the S_3 pH was adjusted to 7.0 or 7.5; these two values did not differ significantly ($p > 0.05$). In this purification step, the supernatant S_3 pH was adjusted to 7.0 using 1.5 M Na_2CO_3 because of the possibility of lowering the number of impurities (21.81 ± 0.86%) present in the purified PA. The loss of PA during the analytical purification process was evaluated after identifying the pH adjustments of supernatants S_2 and S_3 with 1.5 M Na_2CO_3 with the determination of PA in pellets P_1 and P_3 and supernatant S_2 and supernatant S_4 (Fig. 1). The total loss of 1.42 ± 0.03 g PA in 100 g of rice bran represented a 26.88 ± 0.52% loss of PA, considering the content of an initial sample of 5.37 ± 0.09 g of PA in 100 g of rice bran.

Table 5
Purity and yield of purified rice bran phytic acid in rice bran for different pH values of S_3 created with 1.5 M Na_2CO_3.

Supernatant S_3	Purity (%)	Yield (%)
7.0	$78.19^{a} \pm 0.86$	$73.21^{a} \pm 0.90$
7.5	$71.47^{a} \pm 2.95$	$79.09^{a} \pm 4.51$
8.0	$60.89^{b} \pm 6.09$	$80.04^{a} \pm 4.74$
8.5	$58.26^{b} \pm 0.78$	$82.16^{a} \pm 3.87$
9.0	$41.28^{c} \pm 5.30$	$82.73^{a} \pm 7.95$

Mean ± SD ($n = 3$ replicates) followed by different lowercase letters in the same column differ by Tukey test ($p \leq 0.05$).

Table 6
Content of organic, inorganic and total phosphorus, total nitrogen, soluble and total proteins and minerals in standard and purified rice bran phytic acid.

Minerals	Phytic acid samples	
	Standard*	Rice bran PA*
Phytate P ($mg\,g^{-1}$)	$124.86^{a} \pm 2.78$	$64.63^{b} \pm 2.77$
Inorganic P ($mg\,g^{-1}$)	$0.01^{b} \pm 0.00$	$0.11^{a} \pm 0.00$
Total P ($mg\,g^{-1}$)	$132.36^{a} \pm 1.75$	$103.88^{b} \pm 1.17$
Total nitrogen ($g\,100\,g^{-1}$)	$0.03^{b} \pm 0.00$	$0.15^{a} \pm 0.00$
Soluble proteins ($g\,100\,g^{-1}$)	$0.00^{b} \pm 0.00$	$0.60^{a} \pm 0.01$
Total proteins ($g\,100\,g^{-1}$)	$0.19^{b} \pm 0.00$	$0.94^{a} \pm 0.00$
Cu ($mg\,kg^{-1}$)	$0.00^{b} \pm 0.00$	$3.00^{b} \pm 0.00$
Zn ($mg\,kg^{-1}$)	$1.27^{b} \pm 0.00$	$472.19^{a} \pm 0.01$
Ni ($mg\,kg^{-1}$)	$0.95^{b} \pm 0.07$	$1.00^{a} \pm 0.28$
Co ($mg\,kg^{-1}$)	$1.05^{b} \pm 0.07$	$4.15^{a} \pm 0.07$
Mn ($mg\,kg^{-1}$)	$2.45^{b} \pm 0.00$	$820.30^{a} \pm 0.02$
Fe ($mg\,kg^{-1}$)	$1.36^{a} \pm 0.00$	$732.45^{b} \pm 5.45$
Ca ($g\,kg^{-1}$)	$0.01^{b} \pm 0.00$	$1.39^{a} \pm 0.01$
Na ($g\,kg^{-1}$)	$11.00^{a} \pm 0.00$	$11.00^{a} \pm 0.00$
Mg ($g\,kg^{-1}$)	$0.25^{b} \pm 0.00$	$32.73^{b} \pm 0.00$
K ($g\,kg^{-1}$)	$0.00^{b} \pm 0.00$	$1.83^{a} \pm 0.00$

* Mean ± SD ($n = 3$ replicates) followed by different lowercase letters in the same line differ by Tukey test ($p \leq 0.05$).

Therefore, it was confirmed that the technique of extraction and purification of PA was possible with a recovery of 72.88 ± 0.98 g $100\,g^{-1}$ of rice bran, a purity of $78.19 \pm 0.86\%$ and a yield of $73.21 \pm 0.90\%$ (Table 5).

3.3. Evaluation of the analytical procedure for purification of phytic acid in rice bran

The purified PA was characterized to evaluate the efficiency of the procedure (Table 6). The phytic P content of the standard PA was $124.86 \pm 2.78\,mg\,g^{-1}$ and of the purified PA was $64.63 \pm 2.77\,mg\,g^{-1}$, which corresponded to $94.12\,g\,100\,g^{-1}$ and $62.21\,g\,100\,g^{-1}$ of total P. The inorganic P content was $0.01 \pm 0.00\,mg\,g^{-1}$ and $0.11 \pm 0.00\,mg\,g^{-1}$ PA, respectively.

In the purified PA, the total nitrogen content was $0.15 \pm 0.00\%$, the soluble proteins were $0.60 \pm 0.01\%$ and the total protein was $0.94 \pm 0.00\%$. These values were considered low compared to the content of these components in rice bran, which protein content varies from 12.00 to 15.00% (Saunders, 1990), indicating that the protein separation by isoelectric precipitation in this particular process was efficient.

The purified PA showed higher or equal levels of other minerals when compared with the standard PA. The total content of minerals was $1.13\,g\,100\,g^{-1}$ in the standard PA and $3.90\,g\,100\,g^{-1}$ in the purified PA. Mg was the predominant mineral in the purified PA, with $58.31\,g\,100\,g^{-1}$ of total minerals. Sodium accounted for $97.63\,g\,100\,g^{-1}$ and $28.22\,g\,100\,g^{-1}$ of total minerals in the standard and the purified PA, respectively (Table 6). The presence of Na and Mg and other minerals such as K and Ca may indicate a tendency of these minerals to form their respective phytates (Persson et al., 1998). There was a possibility of having formaldehyde residue present at this final phytate preparation however considering this component has the boiling point of −19 °C (WHO, 2006) thus it was volatilized and also the conditions of drying to obtained phytate at 60 °C for 24 h should be enough to eliminate this residue.

The solubility of purified PA was observed at pH 4.0, 6.0 and 8.0 (Fig. 3). It was significantly higher at pH 4.0 ($8.81 \pm 4.83\,mg\,mL^{-1}$), while pH 6.0 and 8.0 (7.45 ± 0.88 and $6.44 \pm 0.41\,mg\,mL^{-1}$) showed no significant difference ($p > 0.05$). The low solubility value of the purified PA at pH 6.0 or 8.0 was possibly due to the presence of other salts, particularly calcium phytates and carbonates associated with zinc and copper phytates, which have a lower solubility at a pH around 6.0 (Nolan et al., 1987). According to Graf et al. (1987), the solubility of PA in different pHs is important for its application as an antioxidant in foods because PA is an effective and nontoxic iron chelator.

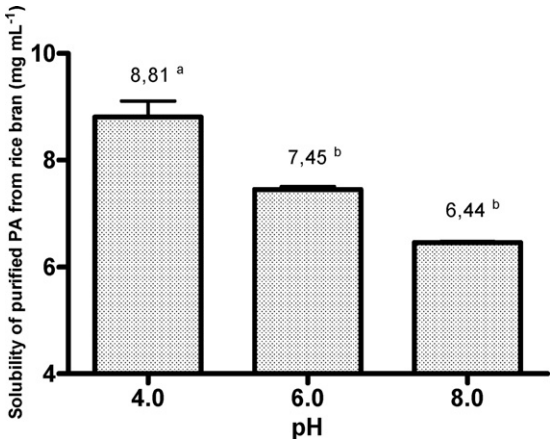

Fig. 3. Solubility of purified rice bran phytic acid at pH 4.0, 6.0 and 8.0. Mean values followed by different lower case letters are significantly different from each other ($p \leq 0.05$) ($n = 3$ replicates).

4. Conclusions

It was possible to establish an improved method for the extraction and purification of PA with high purity and yields. In the procedure for extracting rice bran PA, the temperature, the HCl concentration and the extraction time were significant ($p \leq 0.05$), while the concentration of rice bran ($g\,mL^{-1}$) had no significant effect.

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